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TECHNICAL MANUSCRIPT 590

PRESERVATION OF INSECT CELLS IN LIQUID NITROGEN

Bruce L. Brown
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FEBRUARY 1970

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PRESERVATION OF INSECT CELLS IN LIQUID NITROGEN

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Stanley C. Nagle, Jr.

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Medical Bacteriology Division
BIOLOGICAL SCIENCES LABORATORIES

Project 1R562602AD01

February 1970

ABSTRACT

Aedes aegypti cells grown as suspension cultures and *Aedes albopictus* cells grown as monolayer cultures have been frozen and stored in liquid nitrogen with very little loss of viability. Cultures frozen in medium containing 8% dimethylsulfoxide (DMSO) yielded 93% viable *A. aegypti* cells after storage in liquid nitrogen for 1 month. *A. albopictus* cells preserved similarly yielded a full cell sheet within 4 to 6 days after thawing and were successfully carried through three transfers with no apparent loss of viability.

I. INTRODUCTION*

In the past several years numerous freezing procedures have been described for the preservation of animal cells in vitro; however, published procedures for preservation of cultured insect cells are very limited. This paper describes a method for preserving two strains of insect cells under liquid nitrogen in the presence of dimethylsulfoxide (DMSO).

II. MATERIALS AND METHODS

Aedes aegypti cells** were grown in 250-ml plastic Falcon flasks on the New Brunswick Gyrotory shaker operating at 60 rpm with 25 ml of medium at 26 to 28 C. The media for these cultures were changed weekly by pipetting the cells into a 50-ml conical centrifuge tube and centrifuging for 5 min at 100 rpm. Cells were resuspended in fresh medium and returned to plastic flasks.

Aedes albopictus cells*** were grown statically and formed monolayers in similar flasks containing 25 ml of medium.

Both A. aegypti and A. albopictus cells were grown in the medium developed by Nagle**** (Table 1).

Prior to freezing, the A. aegypti cells were observed in a hemocytometer to determine apparent viability and cell number. When the cell count reached approximately 1.5×10^6 cells per ml, each culture was centrifuged at 1,000 rpm for 5 min and the cells were pooled and resuspended in 10 ml of fresh medium containing various concentrations of DMSO.

The cell suspensions were then dispensed as 1-ml samples into 1.2-ml sterile ampules with a 10-cc hypodermic syringe fitted with a 1½-inch, 20-gauge needle. The ampules were pull-sealed by an automatic ampule sealer.*****

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* This report should not be used as a literature citation in material to be published in the open literature. Readers interested in referencing the information contained herein should contact the senior author to ascertain when and where it may appear in citable form.

** Grace, T.D.C. 1966. Establishment of a line of mosquito (Aedes aegypti L.) cells grown in vitro. Nature 211:366-367.

*** Singh, K.R.P. 1967. Cell cultures derived from larvae of Aedes albopictus (Skuse) and Aedes aegypti (L.). Curr. Sci. 36:506-508.

**** Nagle, Stanley C., Jr. 1969. Improved growth of mammalian and insect cells in media containing increased levels of choline. Appl. Microbiol. 17:318-319.

***** Kahlenberg-Globe Equipment Co., Sarasota, Fla.

TABLE 1. INVERTEBRATE MEDIUM

Component	Concentration, mg/liter
Amino Acids	
L-Alanine	100
L-Arginine·HCl	400
L-Aspartic Acid	300
L-Cysteine·HCl	100
L-Glutamic Acid	400
Glycine	400
L-Histidine·HCl	200
L-Isoleucine	150
L-Leucine	300
L-Lysine·HCl	300
L-Methionine	60
L-Phenylalanine	150
L-Proline	300
L-Serine	150
L-Threonine	75
L-Tryptophan	50
L-Tyrosine	100
L-Valine	150
Heated fetal bovine serum	10%
Streptomycin	100 mg/ml
Penicillin	100 units/ml

Component	Concentration, mg/liter
Salts, etc.	
NaCl	4500
KCl	2500
$CaCl_2 \cdot 2H_2O$	400
$MgCl_2 \cdot 6H_2O$	400
$MgSO_4 \cdot 7H_2O$	400
$NaH_2PO_4 \cdot H_2O$	200
Methylcellulose 15 cps	500
L-Glutamine	200
Glucose	5000
Vitamins	
D-Biotin	1
Choline	50
Folic Acid	1
Niacinamide	1
Ca Pantothenate	2
Pyridoxal·HCl	1
Thiamine·HCl	1
i Inositol	1
Riboflavin	0.1
B_{12}	0.002

Three ampules were immediately opened, centrifuged, and placed into fresh medium containing no DMSO to determine zero-time viability. The remaining ampules were placed in the wire rack of the Linde liquid nitrogen freezer (BF-3, Biological Freezer) and frozen at a rate of 1 degree per min to about -40 C. The frozen ampules were placed immediately in aluminum ampule racks, covered with cardboard tubes* and stored in the Linde liquid nitrogen refrigerator (LNR-35) at -196 C.

The viability of frozen cells was determined by removing three ampules for each sampling period and thawing immediately in a 40 C agitating water bath. Suspensions were completely thawed in 30 to 55 seconds. The ampules were opened aseptically by scoring the neck with a file and snapping off the top. The thawed cell suspensions were aseptically transferred from the ampule to a 50-ml conical centrifuge tube containing 25 ml of fresh growth medium, centrifuged for about 5 min at 1000 rpm, resuspended in 25 ml of fresh medium, examined microscopically, and placed in Falcon flasks. Cell suspensions were placed on the shaker and counted at intervals.

* Frozen Semen Products.

A. albopictus cells grown as monolayers were removed with a 0.25% solution of trypsin, washed in growth medium, resuspended in fresh growth medium containing 8% DMSO, and handled as the A. aegypti cells. These cultures were incubated until full cell sheets developed, then trypsinized and the cell numbers were determined.

III. RESULTS

The data presented in Table 2 show the results obtained when A. aegypti cells were frozen and stored under liquid nitrogen at various concentrations of DMSO. The highest recovery was obtained at concentrations of 8% DMSO; however, satisfactory recovery was also noted at concentrations of DMSO of 4, 6, and 10%. Normal cell growth occurred after incubation of these cultures for 14 days. These results are shown in Figure 1. The peak yields from these recovered cells were 14.7, 16.1, 15.1, 17.6 x 10^5 cells per ml for cells frozen with 4, 6, 8, and 10% DMSO respectively. The excellent growth of cells preserved in 10% DMSO was unexpected in view of these data indicating that 8% DMSO was optimal. We cannot account for this phenomenon; however, all of these growth curves compared favorably with those obtained with unfrozen controls. Concentrations of 12 and 15% DMSO were also used, but at these high levels, the DMSO could not be sufficiently washed from the cells by the method described and the cells showed deterioration after incubation for 4 days.

TABLE 2. VIABILITY OF A. AEGYPTI CELLS STORED WITH DMSO UNDER LIQUID NITROGEN

% DMSO Concentration	% Survival	
	1 Week	1 Month
0	0	0
2	0	0
4	40	47
6	73	60
8	100	93
10	84	70

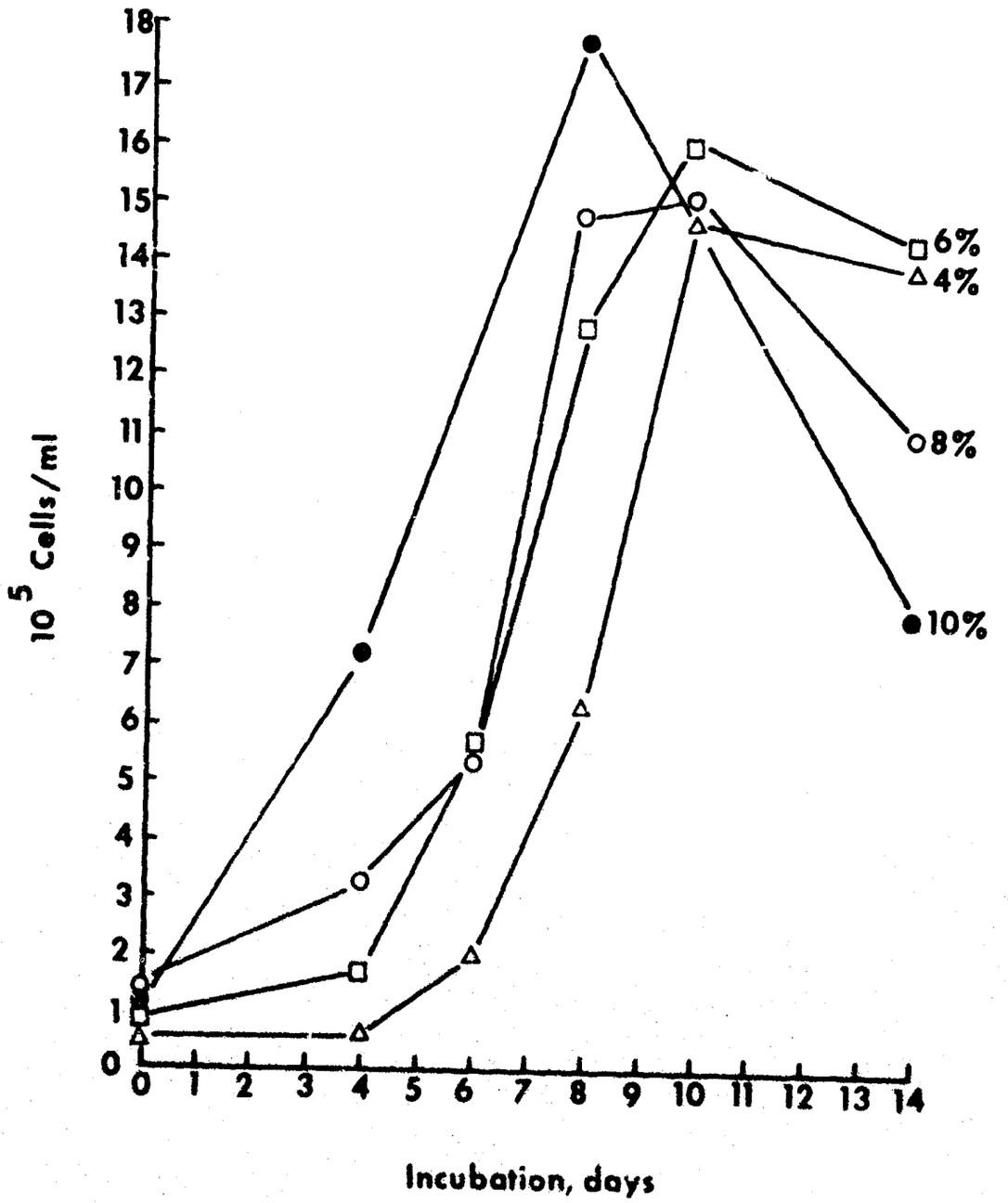

FIGURE 1. Growth of A. aegypti Cells after Storage for 1 Month Under Liquid Nitrogen at 4, 6, 8, and 10% DMSO.

Cells frozen at 2% DMSO and with no DMSO showed no survival at all after storage for as little as 1 week.

The A. albopictus cells that were cultured as monolayers and resuspended in DMSO fluid grew normally when thawed and replaced in fresh medium. A full sheet developed within 6 days, giving about 60×10^6 to 75×10^6 cells per flask from initial inocula of about 10×10^6 per flask. These results have been obtained consistently. Each culture was grown successfully through at least three passages after being thawed.

The ability of the A. albopictus cells to grow well after storage with 8% DMSO indicated that the results obtained with A. aegypti might be generally applicable for some other mosquito cell lines.

In summary, we have shown that it is possible to preserve insect cells frozen under liquid nitrogen in a hemolymph-free medium with DMSO and recover them in excellent condition.

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14. Key Words

Aedes aegypti
Aedes albopictus
Monolayer cultures
Suspension cultures
Preservation
Liquid nitrogen
DMSO

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